

Committee:  
Dr. Cattell, Chm.  
Dr. Bing  
Dr. Jacobson

TOBACCO INDUSTRY RESEARCH COMMITTEE  
150 East Forty Second Street  
New York 17, New York

#223R1

Activated: 7/1/59  
Cf. #128  
Activated: 7/1/56  
Renewed: 7/1/57  
Renewed: 7/1/58

Application for Renewal of Research Grant

Date: February 6, 1960

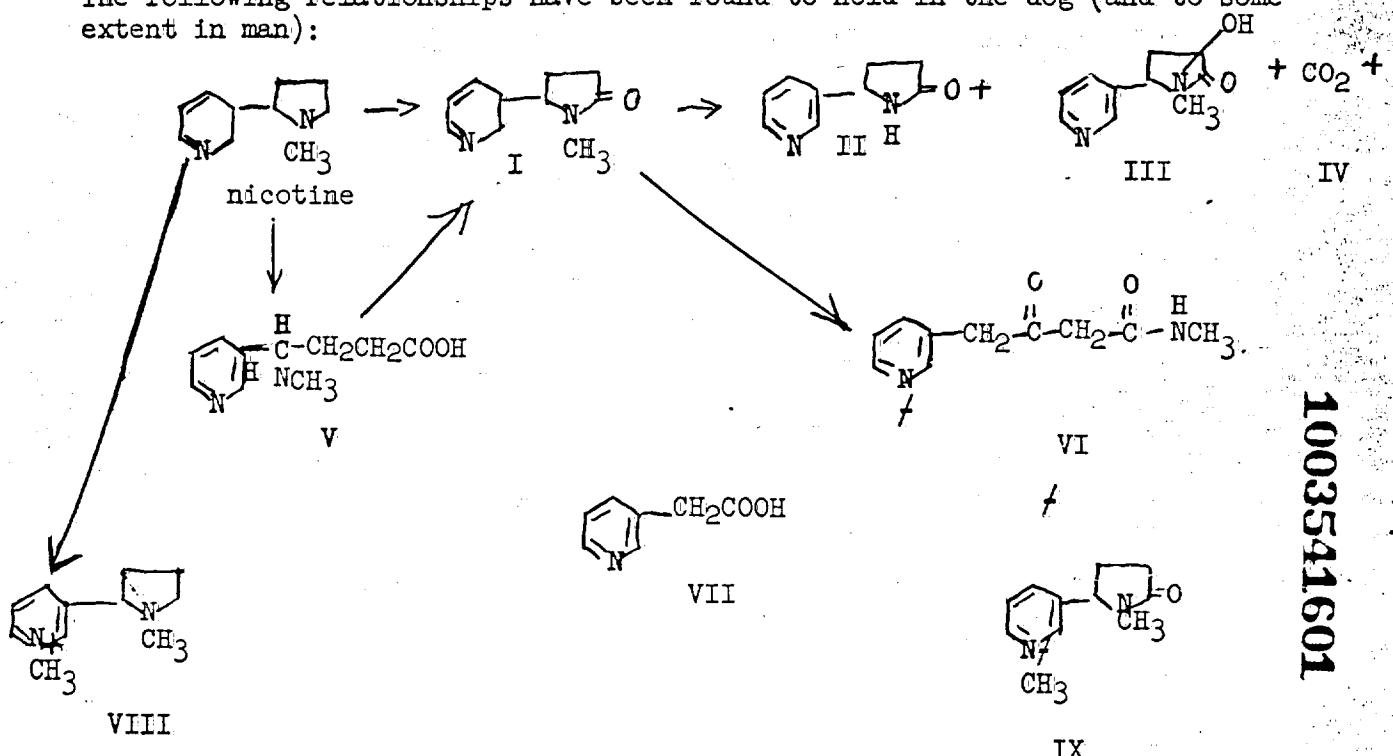
1. Name of Investigator: Dr. Herbert McKennis, Jr. (Dr. Larson)

2. Title: Professor of Pharmacology

3. Institution & Address: Medical College of Virginia  
Richmond 19, Virginia

4. Project or Subject: Enzymatic Transformation of Nicotine and Related Compounds.

5. Detailed Plan of Procedure: As a result of previous TIRC grants, procedures have been developed for the isolation and synthesis of metabolites of nicotine. The following relationships have been found to hold in the dog (and to some extent in man):



The studies have thus far indicated that quaternization of nicotine leading to VIII represents a major metabolic route which may account for as much as ten per cent of the dose and the methylation of cotinine (I) to the product IX is important only when large amounts of cotinine is present in the body. Evidence also indicates the probability that additional quaternary ammonium compounds rise during the metabolism of nicotine.

1003541601

Dr. Herbert McKennis, Jr. (Larson)

February 6, 1960

It becomes desirable to investigate further the quaternary ammonium fraction of urine of nicotine-treated animals in view of the known facility of the body to methylate pyridine rings and the wide spectra of cholinergic and anticholinergic activity which can be anticipated to occur in compounds of this type.

The isolation of 3-pyridylacetic acid (VII) as a result of metabolism of cotinine at once raises the possibility that 3-pyridylacetic acid arises in vivo as a result of the enzymatic cleavage of the metabolite  $\gamma$ -(3-pyridyl)- $\beta$ -oxo-N-methylbutyramide (VI). Studies in vitro indicate that the keto acid from VI has an exceptional stability to both acid and base. This points to the necessity for an enzymatic reaction if the foregoing precursor-metabolite relationship obtains in vivo. A study of the metabolism of both VI and VII appear to hold great promise in helping to account for additional factors in the metabolism of nicotine. This is especially true in the case of 3-pyridylacetic acid (VII) since it has been reported (Ratti and de Fini, The Lancet, 2, 917, 1959) that quantities of 3-pyridylacetic acid in the range of 1.5-2.5 grams per day are tolerated by humans.

It is proposed to continue studies on the metabolism of nicotine in accordance with the foregoing with the general objective of creating a sound foundation which will lead to an eventual understanding of the biological significance of the metabolic events.

6. Budget Plan:

Social Security	426.00
Salaries	20,000.00
Expendable Supplies	4,500.00
Permanent Equipment	7,385.00
Overhead (10%)	3,320.00
Other (Travel)	890.00
Total	\$36,521.00

7. Anticipated Duration of Work: Two years additional. (First annual renewal of the present grant which expires June 30, 1960). Progress will be reported at intervals and in the literature according to custom and past practice.

8. Facilities and Staff Available: General pharmacologic and biochemical equipment, Lardy type Warburg apparatus, refrigerator centrifuge,  $C^{14}$  counting equipment, nuclear instrument glass flow counter with automatic sample changer, paper chromatographic equipment, polarimeter, greenhouse facilities for growing  $C^{14}$  labelled plants, high pressure hydrogenation apparatus. Full time staff: Lennox B. Turnbull, Ph.D., M.S. to be hired, Part time includes: Drs. Herbert McKennis, Jr., Paul S. Larson, H. B. Haag, and J. F. Borzelleca, E. R. Bowman, Public Health Research Fellow and S. Schwarz, graduate student.

9. Additional Requirements: What, if any, will depend upon how project develops.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The American Tobacco Company has, as in the past, provided  $C^{14}$  nicotine. E. R. Bowman has received a fellowship from the National Heart Institute. S. Schwarz expects to receive a fellowship from the National Science Foundation.

1003541602

Dr. Herbert McKennis, Jr. (Larson)

February 6, 1960

The breakdown of salaries is as follows:

Lennox B. Turnbull, Ph.D.	\$9,200.
To be filled M.S.	6,200.
Technician B.S.	3,600.
Animal man part-time	1,000.
	\$ 20,000.

The breakdown on permanent equipment is as follows:

Microelectrophoresis apparatus	1,555.
Actigraph chromatogram canner (with accessories)	2,700.
Water bath shaker	630.
Spectrophotofluorometer (part of cost)	2,500.
	\$ 7,385.

/s/ Herbert McKennis, Jr.  
Director of Project

/s/ W. F. Tompkins  
Business Officer of the Institution

1003541603